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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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EXAMINER	
EINSMANN, J	
ART UNIT	**PAPER NUMBER**
1655	17

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/403,690	Applicant(s) PFEFFER, KLAUS	
	Examiner Juliet C. Einsmann	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 June 2001* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-20* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☒ Claim(s) *3,7 and 13* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *15* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is written in response applicant's correspondence submitted 6/6/2001, paper number 16. Claims 1, 2, 3, 4, 7, 10, 11, 12, 13, 15, and 16 have been amended. Claims 1-20 are pending. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. ~~This action is FINAL.~~

Election/Restrictions

2. The election of species required in paper number 10 is withdrawn in light of applicant's instant amendment of the claims.

Claim Objections

3. Claims 3, 7 and 13 objected to because of the following informalities:

(A) Claim 3 is objected to because it recites "...the primer pair that hybridises **for** the gene encoding heat stabile toxin characteristic of enteroaggregative... (emphasis added)." It appears the "for" should be "to."

(B) Claim 7 is objected to because it recites the misspelling "oligonucleIeotide" in the line describing the pCVD432 plasmid probe.

(C) Claim 13 is objected to because it recites "one probe is included in set of oligonucleotide probes" at the end of the claim. The word "the" should be between "in" and "set."

Appropriate correction is required.

Claim Rejections - 35 USC § 112, 1st paragraph

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claim 16 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. MPEP 2163.06 notes "If new matter is added to the claims, the examiner should reject the claims under 35 U.S.C. 112, first paragraph - written description requirement. In re Rasmussen , 650 F.2d 1212, 211 USPQ 323 (CCPA 1981)."

In the instantly rejected claims, the claiming of a kit appears to represent new matter. This application is a 371 of international application PCT/EP98/02341, which was published as WO98/48046, and it is this original specification which must provide basis for any amendments filed after the original filing date of 4/21/98. No specific basis for this limitation was identified in the applicant's response, nor did a review of the specification by the examiner find any basis for the limitation. Since no basis has been identified, the claims are rejected as incorporating new matter.

Response to Remarks

Applicant's arguments have been considered but are not persuasive. While the specification is not required to literally support each word in a claim, the specification should conceptually support the claimed subject matter. Applicant has not presented support for there is no description of a kit in the specification, and further, the word "kit" is not supported by any

clear definition or meaning. That the word "kit" clearly is intended to provide some meaning in the claims which differentiates kits from mere sets of primers and probes. This is evidenced by the fact that there are "set" claims and there is a "kit" claim in the instant claim set. This meaning has not been discussed in the instant specification either literally or conceptually. In the instant case, the specification discusses sets of primers and probes but does not address kits which comprise these sets primers and probes. Due to the absence of any discussion of "kits" in the instant specification, this rejection is maintained.

Claim Rejections - 35 USC § 112, 2nd Paragraph

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 1-12 and 16-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-9 and 17-20 are indefinite because the preamble of claim 1 recites "A Polymerase Chain Reaction (PCR) method for detection and differentiation of pathogenic enterobacteria in a sample," but the claim does not contain any process steps which require PCR or which indicate how the method accomplishes "detection and differentiation" of the enterobacteria.

Claims 1-9 and 17-20 are also indefinite over the recitation of "a set of oligonucleotide primer pairs is added to said sample, each primer pair being capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative,

enteroinvasive, enteropathogenic, and enterohemorrhagic *E. coli* and wherein for amplification of each subgroup at least one primer pair is added to the sample" because it is not clear how many primer pairs this claim is requiring in the "set of oligonucleotide primer pairs." A "set of primer pairs" could comprise only two primer pairs, and the claim seems to read on a method which utilizes only two primer pairs provided that each of these primer pairs is capable of amplifying a pathogenic *E. coli* in one of the listed subgroups, and provided that for amplification of each of these two subgroups one primer is added to the sample. However, from the arguments provided by Applicant, it appears that Applicant interprets the claim so as to require a set of primer pairs which includes primers that are capable of amplifying each subgroup. This limitation is not clear from the language of the claim, and the claim should be amended to clarify this position.

Claims 2-3 are further indefinite because claim 2 recites the improper Markush language "selected from." Amendment of the claim to recite "selected from the group consisting of" would obviate this rejection.

Claims 4-8 are indefinite because the phrases "the amplification of DNA" and "the target DNA" and "the amplification process" lack proper antecedent basis in claim 1. Claim 1 refers to a PCR process in its preamble, but claim 1 does not contain steps which recite the amplification of DNA, target DNA or an amplification process. Furthermore, "said pol" in the last line of claim 4 lacks proper antecedent basis, because claim 4 previously recites a "polymerase" in line 1 of claim 4, but it does not recite a "pol."

Claims 4-8 are further indefinite because claim 4 recites the use of only a single probe, yet claim one requires, at the very least, the amplification of at least two DNA sequences, each

specific to a different subgroup of *E. coli*. Therefore, it is unclear if claim 4 intends for only one probe to be included in the method or more than one probe.

Claim 5 is indefinite because it refers to "the respective virulence factor/toxin gene" to be detected, yet the independent claim from which it depends requires the detection of more than one virulence/toxin gene.

Claim 6 indefinite because it appears to require that the same labeled oligonucleotide probe be specific for the detection of nine different characteristics, since each section of the claim recites "the labeled oligonucleotide probe" and there is no designation in the claim that these probes are meant to be alternatives. Furthermore, it is noted that none of the previous claims require the amplification of target DNA for each and every one of the "characteristics" listed in claim 6.

Claims 6, 7 and 14 are indefinite over the recitation of "specific for the detection of heat labile toxin (or any other toxin)" because the probes are actually specific for the detection of the gene encoding the toxin, not the toxin.

Claim 7 is indefinite insomuch as it depends from claim 6 and it is not clear how many or which probes are required for use in the recited methods.

In claim 8, the recitation "fluorescent reporter dye" lacks proper antecedent basis because neither claim 1 nor claim 4 from which claim 8 depends specifically recites a fluorescent reporter dye.

Claim 9 is indefinite because the phrase "the amplification process" lacks proper antecedent basis in claim 1. Claim 1 refers to a PCR process in its preamble, but claim 1 does not contain steps which recite the amplification of DNA, target DNA or an amplification process.

In the last line of claim 10, the phrase "said set of oligonucleotide probes" lacks proper antecedent basis because the claim does not previously recite a set of oligonucleotide probes.

Claims 10-12 and 16 are indefinite over the recitation of "each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic, and enterohemorrhagic *E. coli* and wherein for amplification of each subgroup at least one primer pair is included in said set of oligonucleotide probes" because it is not clear how many primer pairs this claim is requiring in the "set of oligonucleotide primer pairs." A "set of primer pairs" could comprise only two primer pairs, and the claim seems to read on a method which utilizes only two primer pairs provided that each of these primer pairs is capable of amplifying a pathogenic *E. coli* in one of the listed subgroups, and provided that for amplification of each of these two subgroups one primer is added to the sample. However, from the arguments provided by Applicant, it appears that Applicant interprets the claim so as to require a set of primer pairs which includes primers that are capable of amplifying each subgroup. This limitation is not clear from the language of claim 10.

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. Claim 13 is rejected under 35 U.S.C. 102(b) as being anticipated by Levine *et al.* (American Journal of Epidemiology (1993 Nov 15) 138(10)849-869).

Levine *et al.* teach a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggregative *E. coli* in a sample, wherein for each subgroup of pathogenic *E. coli* a different probe is included in the set. The set of probes taught by probes for the detection of the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 1, 2, 9, 10, 17, and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* (American Journal of Epidemiology, Nov. 15, 1993, 138(10): 849-869) in view of Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149).

Levine *et al.* teach a method for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein the sample is human fecal matter. Levine *et al.* specifically teach the use of oligonucleotide

probes to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a method that utilizes PCR amplification of the target region.

Lang *et al.* teach a method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs that allow the differentiation of enterotoxigenic and enterohemorrhagic E. coli strains by amplification of a toxin gene characteristic for the respective group of the pathogenic E. coli strains (p. 3146-3147). The LT (heat-labile) gene was used to detect enterotoxigenic E. coli, and the SLT I and SLT II genes were used to detect enterohemorrhagic E. coli. For use in their methods, Lang *et al.* a set of primers appropriate for their amplification method and a set of labeled probes useful to detect the amplified products (p. 3146).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine *et al.* so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang *et al.* in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes. Lang *et al.* teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method.

With regard to the specific PCR parameters recited in claim 9, these are considered to be parameters obtained by routine optimization of an assay. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the PCR parameters claimed performed are other than routine, that the methods resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. As such, in the absence of a secondary consideration, such as unexpected results, these are considered obvious over the art of record.

12. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* as applied to claims 1, 2, 9, 10, 17, and 18 above and further in view of Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733), Paton *et al.* (GenBank Z36899) and of Hogan *et al.* (US 5595874).

The teachings of Levine *et al.* in view of Lang *et al.* are applied herein as discussed above.

Levine *et al.* in view of Lang *et al.* do not teach methods or sets of primers and probes which include instant SEQ ID NO: 1, 2, or 19 or instant SEQ ID NO: 15, 16 or 26.

Yamamoto *et al.* teach the nucleotide sequence of heat labile toxin of E. coli pathogenic for humans (Figure 2). Instant SEQ ID NO: 1 consists of nucleotides 49-70 of this sequence, instant SEQ ID NO: 2 consists of the complement of nucleotides 367-388 of this sequence, and

instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* on page 730.

Paton *et al.* (GenBank Z36899, May 1995) teach the nucleotide sequence of the shiga-like toxin SLTI gene. Instant SEQ ID NO: 15 consists of nucleotides 1113-1135 of this sequence, instant SEQ ID NO: 15 consists of the complement of nucleotides 1376-1400 of this sequence, and instant SEQ ID NO: 26 consists of the complement of nucleotides 1338-1367 of the sequence taught by Paton *et al.*

Hogan et al. (US 5595874) teach the use of specific primers and furthermore provides specific guidance for the selection of primers,

> "At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
>
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided (Col. 6, line 50-Col. 7, line 13)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have substituted primers and probes taken from the sequences taught by

Yamamoto *et al.* and Paton *et al.* in the methods taught by Levine *et al.* in view of Lang *et al.* The ordinary practitioner would have been motivated to make such a substitution in order to have provided a method that is a functional equivalent of the method taught by Lang *et al.* in that both methods would be useful for the detection of enterotoxigenic E. coli. The teachings of Hogan *et al.* would have provided adequate direction to the ordinary practitioner in the selection of the probes and primers from the sequence taught by Yamamoto *et al.* or Paton *et al.* In the absence of secondary considerations, such as unexpected results, the substitution of probes and primers selected using the guidance provided by Hogan *et al.* and the sequence provided by Yamamoto *et al.* and Paton *et al.* into the methods taught by Levine *et al.* in view of Lang *et al.* are obvious in view of the prior art.

13. Claims 4, 5, 6, and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over as being unpatentable over Levine *et al.* in view of Lang *et al.* as applied to claims 1, 2, 9, 10, 17, and 18 above and further in view of in view of Livak *et al.* (PCR Methods and Applications (1995) 4:357-362).

The teachings of Levine *et al.* in view of Lang *et al.* are applied to this rejection as discussed in the rejection of claims 1, 2, 9, 10, 17, and 18 above.

Levine *et al.* in view of Lang *et al.* do not teach methods wherein a polymerase having 5'-3' exonuclease activity is used for the amplification of DNA and a probe labeled at both ends is used to detect amplified samples.

Livak *et al.* teach a PCR-based assay that uses the hydrolysis of an internal fluorogenic probe to monitor the amplification of the target (ABSTRACT). The method taught by Livak *et*

al. utilizes a polymerase having 5'-3; exonuclease activity and a probe labeled at the 5' end with the fluorescent dye 6-carboxyfluorescein (6-FAM) and also labeled at the 3' end with the fluorescent quencher dye 6-carboxytetramethyl-rhodamine(TAMARA) (Fig. 2). The labeled probe hybridizes with the target DNA and is included in the amplification process.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have combined the methods taught by Levine *et al.* in view of Lang *et al.* with those taught by Livak *et al.* The ordinary practitioner would have been motivated to use an assay such as the one taught by Livak *et al.* for the detection of E. coli since Livak *et al.* teach that such a method is a homogenous assay for detecting the accumulation of specific PCR products and probes with a label attached to the 5' end and a quencher at the 3' end exhibit a larger signal in the 5' nuclease PCR assay than internally labeled probes (Abstract).

14. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* in view of Livak *et al.* as applied to claims 4, 5, 6, and 8 above, and further in view of Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733), Paton *et al.* (GenBank Z36899) and of Hogan *et al.* (US 5595874).

The teachings of Levine *et al.* in view of Lang *et al.* in view of Livak *et al.* are applied to claim 7 as they are applied to claims 4-6 and 8 above. Levine *et al.* in view of Lang *et al.* in view of Livak *et al.* do not teach methods in which the probe used to detect the LT gene is SEQ ID NO: 19 or in which the probe used to detect the SLTI gene is SEQ ID NO: 26.

Yamamoto *et al.* teach the nucleotide sequence of heat labile toxin of E. coli pathogenic for humans (Figure 2). Instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* on page 730.

Paton *et al.* (GenBank Z36899, May 1995) teach the nucleotide sequence of the shiga-like toxin SLTI gene. Instant SEQ ID NO: 26 consists of the complement of nucleotides 1338-1367 of the sequence taught by Paton *et al.*

Hogan et al. (US 5595874) teach the use of specific primers and furthermore provides specific guidance for the selection of primers,

> "At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
>
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided (Col. 6, line 50-Col. 7, line 13)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have substituted primers and probes taken from the sequence taught by Yamamoto *et al.* in the methods taught by Levine *et al.* in view of Lang *et al.* in view of Livak *et*

al. The ordinary practitioner would have been motivated to make such a substitution in order to have provided a method that is a functional equivalent of the method taught by Levine *et al.* in view of Lang *et al.* in view of Livak *et al.* in that both methods would be useful for the detection of enterotoxigenic E. coli. The teachings of Hogan *et al.* would have provided adequate direction to the ordinary practitioner in the selection of the probes and primers from the sequence taught by Yamamoto *et al.* In the absence of secondary considerations, such as unexpected results, the substitution of probes and primers selected using the guidance provided by Hogan *et al.* and the sequence provided by Yamamoto *et al.* into the methods taught by Levine *et al.* in view of Lang *et al.* in view of Livak *et al.* are obvious in view of the prior art.

15. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* as applied to claims 1, 2, 8, 10, 17 and 18 above, and further in view of Savarino *et al.* (PNAS USA, Vol. 90, pp. 3093-3097 (1993)).

The teachings of Levine *et al.* in view of Lang *et al.* are applied to claim 11 as they are applied above. Levine *et al.* further teach that the EAggEC plasmid which is the target of one of their probes encodes the EAggEC heat stable enterotoxin (p. 854).

Levine *et al.* in view of Lang *et al.* do not teach a method utilizes a primer set which would hybridize to a gene encoding heat stable toxin of enteroaggregative *E. coli*.

Savarino *et al.* teach that some EAggEC produce a heat stable enterotoxin named EAST1. Savarino *et al.* provide the DNA sequence of the gene encoding the toxin, and they provide a PCR amplification assay for the amplification the gene (p. 3094 and Fig. 2).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have included a primer pair designed to amplify a portion of the EAST1

gene in the detection method taught by Levine *et al.* in view of Lang *et al.* With such an inclusion, the method taught by Levine *et al.* in view of Lang *et al.* in view of Savarino *et al.* would utilize a set of primers which comprises a pair of primers that hybridizes to a gene encoding heat stable toxin of EaggEC. The ordinary practitioner would have been motivated to have included the additional primer pair set in order to have utilized yet another tool for the detection of pathogenic *E. coli* in a sample, thus providing a more comprehensive assay for the detection of diarrhea causing *E. coli.* Therefore, in the absence of a secondary consideration, such as an unexpected result, the claimed invention is obvious over the prior art.

16. Claim 14 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of both Savarino *et al.* (PNAS USA (1993) 90:3093-3097) and Louie *et al.* (Epidemiol. Infect. (1994), 112:449-461).

Levine *et al.* teach a set of probes for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein for each subgroup of pathogenic *E. coli* a different probe is included in the set. The set of probes taught by probes for the detection of the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a set of probes comprising a probes specific for heat stable characteristic for EaggEC or the eae gene.

Savarino *et al.* teach that some EAggEC produce a heat stable enterotoxin named EAST1. Savarino *et al.* provide the DNA sequence of the gene encoding the toxin, and they provide a PCR amplification assay for the amplification the gene (p. 3094 and Fig. 2). Savarino *et al.* further teach the use of the PCR product produced by their method as a probe in southern blot analysis (p. 3094).

Louie *et al.* teach PCR assays and probes that allow the specific identification of EPEC which contain the *eae* gene (Table 2; p. 452-453). Louie *et al.* teach that their methods "will be useful methods for subclassification of EPEC and VTEC (p. 459)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have included probes to the EaggEC heat stable enterotoxin gene and the eae gene in the probe set taught by Levine *et al.* The ordinary practitioner would have been motivated to provide a set comprising all of these probes in order to have provided a set of probes which is useful for the detection and differentiation of many different types of toxigenic *E. coli*.

17. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of both Savarino *et al.* (PNAS USA (1993) 90:3093-3097) and Louie *et al.* (Epidemiol. Infect. (1994), 112:449-461) as applied to claim 14 above, and further in view of all of the following: Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733), Moseley *et al.* (GenBank M34916), Yamamoto *et al.* (Infection and Immunology (1996) 64 (4): 1441-1445), Schmidt *et al.* (Journal of Clinical Microbiology (1995) 33(3): 701-705), Lampel *et al.* (US 5041372), Franke *et al.* (Journal of Clinical Microbiology (1994) 32(10):2460-2463),

Kaper (1995, GenBank Accession Z11541), Paton *et al.* (GenBank Z36899), and Paton *et al.* (GenBank L11079).

The teachings of Levine *et al.* in view of Savarino *et al.* and Louie *et al.* are applied to this claim as discussed above in the rejection of claim 14. Levine *et al.* in view of Savarino *et al.* and Louie *et al.* do not provide probes consisting of the specific sequences recited in claim 15.

However, the full length sequences of all of genes from which the probes used in the methods of Levine *et al.*, Savarino and Louie *et al.* were derived were known in the prior art at the time the invention was made.

Instant SEQ ID NO: 19 consists of nucleotides 78-104 of the sequence taught by Yamamoto *et al.* (1983) on page 730.

Instant SEQ ID NO: 20 consists of the complement of nucleotides 306-334 of the sequence taught by Moseley *et al.*

Instant SEQ ID NO: 21 consists of nucleotides 30-69 of the EAST 1 sequence taught by Yamamoto *et al.* (1996) in figure 4.

Instant SEQ ID NO: 22 consists of the complement of nucleotides 639-668 of the pCVD432 plasmid sequence taught in figure 1 of Schmidt *et al.*

Instant SEQ ID NO: 23 consists of the complement of nucleotides 177-202 of the probe taught by Lampel *et al.* (Col. 13-14).

Instant SEQ ID NO: 24 consists of nucleotides 574-601 of the EAF probe taught by Franke *et al.*

Instant SEQ ID NO: 25 consists the complement of nucleotides 908-935 of the sequence for the eae gene disclosed in GenBank Z11541.

Instant SEQ ID NO: 26 consists of the complement of nucleotides 1338-1367 of the sequence taught by Paton *et al.*

The specific probes of the instant invention represent structural and functional homologues of the longer probes taught in the prior art. That is, the probes recited in claim 15 would be expected to detect the larger sequences of which they are fragments, much like the probes taught in Levine *et al.*, Savarino *et al.* and Louie *et al.* In the recent court decision *In Re Deuel* 34 USPQ 2d 1210 (Fed. Cir. 1995), the court determined that the existence of a general method of identifying a specific DNA does not make the specific DNA obvious. Regarding structural or functional homologues, however, the court stated

> "Normally, a *prima facie* case of obviousness is based upon structural similarity, i.e., an established structural relationship between a prior art compound and the claimed compound. Structural relationships may provide the requisite motivation or suggestion to modify known compounds to obtain new compounds. For example, a prior art compound may suggest its homologues because homologues often have similar properties and therefore chemists of ordinary skill would ordinarily contemplate making them to try to obtain compounds with improved properties."

Since the claimed oligonucleotides simply represent structural homologues of the full length disclosed sequences and the probes disclosed, a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed probes are *prima facie* obvious over the cited reference in the absence of secondary considerations.

18. Claim 16 is rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* and further in view of the Stratagene Catalog.

Levine *et al.* teach a method for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC),

enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein the sample is human fecal matter. Levine *et al.* specifically teach the use of oligonucleotide probes to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a method that utilizes PCR amplification of the target region.

Lang *et al.* teach a method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs that allow the differentiation of enterotoxigenic and enterohemorrhagic E. coli strains by amplification of a toxin gene characteristic for the respective group of the pathogenic E. coli strains (p. 3146-3147). The LT (heat-labile) gene was used to detect enterotoxigenic E. coli, and the SLT I and SLT II genes were used to detect enterohemorrhagic E. coli. For use in their methods, Lang *et al.* a set of primers appropriate for their amplification method and a set of labeled probes useful to detect the amplified products (p. 3146). Furthermore, Lang *et al.* teach a set of internal oligonucleotide probes for the detection of PCR products using a Southern Blot (p. 3146).

Levine *et al.* in view of Lang *et al.* do not teach kits.

Stratagene teaches gene characterization kits. The ordinary practitioner would have been motivated to have produced such a kit because since the Stratagene catalog expressly teaches the benefits to the practitioner of kits:

> "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually more expensive for a

> small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, pre-mixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control."

Therefore, the kits of the instant claims are *prima facie* obvious over the disclosure of Lang *et al.* in view of the Stratagene catalog.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine *et al.* so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization probes. The ordinary practitioner would have been motivated by the success of Lang *et al.* in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes. Lang *et al.* teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method.

Further, in view of the teachings of the Stratagene Catalog, the ordinary practitioner would have been motivated to provide the reagents necessary to complete this assay in a kit. Therefore, the kit of the instant claim is *prima facie* obvious over the disclosure of Lang *et al.* in view of Livak *et al.* and further in view of the Stratagene catalog.

19. Claims 17, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Levine *et al.* in view of Lang *et al.* in view of Tsen *et al.* (Journal of Food Protection (1996) Vol. 59, No. 8, pp. 795-802, ABSTRACT ONLY).

Levine *et al.* teach a method for the detection and differentiation of pathogenic enterobacteria in a sample which comprises the detection and differentiation of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EaggEC) in a sample, wherein the sample is human fecal matter. Levine *et al.* specifically teach the use of oligonucleotide probes to the heat labile (LT) and heat stable (ST) genes of ETEC, the invasiveness plasmid (inv) of EIEC, the EPEC Adherence Factor (EAF) plasmid of EPEC, the shiga-like toxin genes sltI and sltII of EHEC, and the EaggEC plasmid (pCVD432) of EaggEC (see p. 854).

Levine *et al.* do not teach a method that utilizes PCR amplification of the target region.

Lang *et al.* teach a method for detection of pathogenic enterobacteria in a sample comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs that allow the differentiation of enterotoxigenic and enterohemorrhagic E. coli strains by amplification of a toxin gene characteristic for the respective group of the pathogenic E. coli strains (p. 3146-3147). The LT (heat-labile) gene was used to detect enterotoxigenic E. coli, and the SLT I and SLT II genes were used to detect enterohemorrhagic E. coli. For use in their methods, Lang *et al.* a set of primers appropriate for their amplification method and a set of labeled probes useful to detect the amplified products (p. 3146). Lang *et al.* further teach that both ETEC and EHEC have been associated with the ingestion of food and are both known to be pathogenic to humans, causing symptoms including diarrhea.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Levine *et al.* so as to have included a PCR step for the amplification of the target DNA prior to the utilization of the hybridization

probes. The ordinary practitioner would have been motivated by the success of Lang *et al.* in developing such a PCR method for the detection of pathogenic enterobacteria, and the ordinary practitioner would have been motivated to include an amplification step in the methodology taught by Lang *et al.* in order to have provided a larger quantity of target DNA for the probes. Lang *et al.* teach that their PCR method provides the advantage of rapidly detecting E. coli in a sample, and thus the ordinary practitioner would have been further motivated to use such a method.

Levine *et al.* in view of Lang *et al.* do not teach methods in which these pathogenic bacteria are detected in consumables.

However, methods for the detection of pathogenic bacteria in consumables such as milk were routine in the art at the time the invention was made. Such a method for the detection of the LT gene of E. coli in milk is exemplified by Tsen *et al.* (ABSTRACT).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have used a method such as the one taught by Lang *et al.* in order to detect the presence of know pathogenic organisms in milk. The ordinary practitioner would have been motivated to create such a method in order to provide a method for the screening milk samples for possible E. coli infections.

Response to Remarks

Applicant's remarks primarily directed to the amended claims. The new grounds of rejection listed above address these remarks.

Conclusion

20. Claim 12 is free of the prior art. Claim 12 requires, among other things, a pair of nucleic acid primers directed to SEQ ID NO: 5 and 6. The prior art does not teach or suggest such a primer pair.

21. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C. Einsmann whose telephone number is (703) 306-5824. The examiner can normally be reached on Monday through Thursday, 7:00 AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones can be reached on (703) 308-1152. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 and (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Juliet C. Einsmann
Examiner
Art Unit 1655

August 23, 2001

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600

8/27/01